L2: Entry 12 of 19 File: USPT Jan 17, 1995

US-PAT-NO: 5382518
DOCUMENT-IDENTIFIER: US 5382518 A

TITLE: Urate oxidase activity protein, recombinant gene coding therefor, expression vector, micro-organisms and transformed cells

DATE-ISSUED: January 17, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Caput; Daniel	Toulouse			FR
Ferrara; Pascual	Villefranche de Lauragais			FR
Guillemot; Jean-Claude	Toulouse			FR
Kaghad; Mourad	Ramonville St. Agne			FR
Legoux; Richard	Caraman			FR
Loison; Gerard	Toulouse			FR
Larbre; Elisabeth	Avignon			FR
Lupker; Johannes	Castanet-Tolosan			FR
Leplatois; Pascal	Cuq Toulza			FR
Salome; Marc	Castanet-Tolosan			FR
Laurent; Patrick	Pechbusque			FR

US-CL-CURRENT: 435/191; 435/69.1

CLAIMS:

from A. Flavus

What is claimed is:

1. A protein possessing a specific urate oxidase activity of at least 16 U/mg and having the following sequence (SEQ ID NO:1):

Ser	Ala	Val	Lys	Ala	Ala	Arg	Tyr	Gly	Lys
Asp	Asn	Val	Arg	Val	Tyr	Lys	Val	His	Lys
Asp	Glu	Lys	Thr	Gly	Val	Gln	Thr	Val	Tyr
Glu	Met	Thr	Val	Cys	Val	Leu	Leu	Glu	Gly
Glu	Ile	Glu	Thr	Ser	Tyr	Thr	Lys	Ala	Asp
Asn	Ser	Val	Ile	Val	Ala	Thr	Asp	Ser	Ile
Lys	Asn	Thr	Ile	Tyr	Ile	Thr	Ala	Lys	Gln
Asn	Pro	Val	Thr	Pro	Pro	Glu	Leu	Phe	Gly
Ser	Ile	Leu	Gly	Thr	His	Phe	Ile	Glu	Lys
Tyr	Asn	His	Ile	His	Ala	Ala	His	Val	Asn
Ile	Val	Cys	His	Arg	Trp	Thr	Arg	Met	Asp
Ile	Asp	Gly	Lys	Pro	His	Pro	His	Ser	Phe

Ile	Arg	Asp	Ser	Glu	Glu	Lys	Arg	Asn	Val
Gln	Val	Asp	Val	Val	Glu	Gly	Lys	Gly	Ile
Asp	Ile	Lys	Ser	Ser	Leu	Ser	Gly	Leu	Thr
Val	Leu	Lys	Ser	Thr	Asn	Ser	Gln	Phe	Trp
Gly	Phe	Leu	Arg	Asp	Glu	Tyr	Thr	Thr	Leu
Lys	Glu	Thr	Trp	Asp	Arg	Ile	Leu	Ser	Thr
Asp	Val	Asp	Ala	Thr	Trp	Gln	Trp	Lys	Asn
Phe	Ser	Gly	Leu	Gln	Glu	Val	Arg	Ser	His
Val	Pro	Lys	Phe	Asp	Ala	Thr	Trp	Ala	Thr
Ala	Arg	Glu	Val	Thr	Leu	Lys	Thr	Phe	Ala
Glu	Asp	Asn	Ser	Ala	Ser	Val	Gln	Ala	Thr
Met	Tyr	Lys	Met	Ala	Glu	Gln	Ile	Leu	Ala
Arg	Gln	Gln	Leu	Ile	Glu	Thr	Val	Glu	Tyr
Ser	Leu	Pro	Asn	Lys	His	Tyr	Phe	Glu	Ile
Asp	Leu	Ser	Trp	His	Lys	Gly	Leu	Gln	Asn
Thr	Gly	Lys	Asn	Ala	Glu	Val	Phe	Ala	Pro
Gln	Ser	Asp	Pro	Asn	Gly	Leu	Ile	Lys	Cys
Thr	Val	Gly	Arg	Ser	Ser	Leu	Lys	Ser	Lys
Leu									

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preceded, if appropriate, by a methionine.

2. A protein according to claim 1, wherein said protein is produced by recombinant methods.

3. A protein according to claim 1, which represents, by analysis on a bidimensional Laemmli/SDS-Agarose gel, a spot of molecular mass of about 33.5 kDa, representing at least 90% of the protein mass.

4. A protein according to claim 1, having a purity degree, determined by liquid chromatography on a C8 grafted silica column, higher than 80%.

5. A protein according to claim 1, having an isoelectric point around 8.0.

6. A protein according to claim 1, which carries a blocking group on the amino-terminal serine.

7. A pharmaceutical composition comprising a protein according to claim 1.

8. A protein according to claim 6, wherein said blocking group is an acetyl group.

9. A protein according to claim 2, possessing a specific urate oxidase activity of about 30 U/mg.

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L5: Entry 1 of 1 File: USPT Sep 22, 1998

DOCUMENT-IDENTIFIER: US 5811096 A
**** See image for Certificate of Correction ****
TITLE: Stable liquid composition containing urate oxidase and lyophilized composition for its preparation

Brief Summary Text (4):
Urate oxidase is a tetramer enzyme composed of four identical units with a molecular weight of 34,152. Each monomer unit, formed from a single polypeptide chain containing 301 amino acids, is acetylated at the N-terminal end and does not have disulphide bridges. The optimum pH for stability of the enzymatic activity of urate oxidase in solution is pH=8 (Bayol A. et al., accepted for publication Biophys. Chem., 1995 (54), 229-35).

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L2: Entry 10 of 19 File: USPT Jul 30, 1996

US-PAT-NO: 5541098
DOCUMENT-IDENTIFIER: US 5541098 A

TITLE: Urate oxidase activity protein, recombinant gene coding therefor, expression vector, micro-organisms and transformed cells

DATE-ISSUED: July 30, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Caput; Daniel	Toulouse			FR
Ferrara; Pascual	Vilefranche de Lauragais			FR
Guillemot; Jean-Claude	Toulouse			FR
Kaghad; Mourad	Ramonville St. Agne			FR
LeGoux; Richard	Caraman			FR
Loison; G erard	Toulouse			FR
Larbre; Elisabeth	Avignon			FR
Lupker; Johannes	Castanet-Tolosan			FR
Leplatois; Pascal	Cuq Toulza			FR
Salome; Marc	Castanet-Tolosan			FR
Laurent; Patrick	Pechbusque			FR

US-CL-CURRENT: 435/191; 435/252.33, 435/254.21, 435/320.1, 435/365, 536/23.2

CLAIMS:

What is claimed is:

1. An isolated, purified gene which comprises a polynucleotide encoding the protein of the sequence of SEQ ID NO:2.

2. An isolated, purified gene according to claim 1, which permits expression in prokaryotic microorganisms.

3. An isolated, purified gene according to claim 12, wherein the polynucleotide has the sequence of SEQ ID NO:3.

4. An isolated, purified gene according to claim 1, which permits expression in eukaryotic cells.

5. An isolated, purified gene according to claim 4, wherein the polynucleotide has the sequence of SEQ ID NO:4.

6. An isolated, purified gene according to claim 1, which permits expression in animal cells.

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=> s protein concentration and aggregation
L1         1416 PROTEIN CONCENTRATION AND AGGREGATION

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PROCESSING COMPLETED FOR L1
L2          646 DUP REM L1 (770 DUPLICATES REMOVED)

=> focus l2 1-10 ibib ab
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L3          646 FOCUS L2 1-

=> d l3 1-10 ibib ab

L3   ANSWER 1 OF 646  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:        1980:512528  HCAPLUS
DOCUMENT NUMBER:         93:112528
TITLE:                   Protein concentration dependence
                         on aggregation behavior and properties of
                         soybean 7S and 11S globulins during alkali-treatment
AUTHOR(S):               Ishino, Keizo; Kudo, Shiro
CORPORATE SOURCE:        Food Res. Lab., Asahimatsu Kori-Tofu Co., Ltd., Iida,
                         399-25, Japan
SOURCE:                  Agricultural and Biological Chemistry (1980), 44(6),
                         1259-66
                         CODEN: ABCHA6; ISSN: 0002-1369
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   The protein concn. dependent changes in aggregation and phys.
     properties of alkali-treated soybean 7 S and 11 S globulins were
     investigated by viscosity, electrophoresis, CD, pulsed NMR, emulsion
     capacity, and CaCl2 pptn. measurements.  At lower protein concns. the
     intrinsic viscosity decreased and the fractions penetrating
     electrophoresis gel increased.  The reduced contacts of proteins during
     neutralization resulted in smaller aggregates.  Specific fractions that
     were more sensitive to protein concn. on aggregation were obsd.
     for 11 S globulin.  The quantity of bound water depended only on the pH at
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the 7% concn. treatment. When the gel was formed, the bound water of protein increased, e.g., 0.085 and 0.135 g/g protein at pH 10.6 and 13.2 treatment, resp., whereas at 1% treatment, bound water showed almost no pH dependence (about 0.13 g/g protein). Proteins prepd. at higher protein concns. were characterized by higher emulsion capacity and CaCl2 pptn. ability. No protein concn. dependence was seen in the secondary structure of the aggregates.

L3 ANSWER 2 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:478739 HCAPLUS
DOCUMENT NUMBER: 125:193943
TITLE: Effects of **protein concentration** and degree of hydrolysis during heating on the **aggregation** of .beta.-lactoglobulin
AUTHOR(S): Sato, K.; Imai, H.; Nakamura, Michiko; Nishiya, T.; Kawanari, M.; Nakajima, I.
CORPORATE SOURCE: Tech. Res. Inst., Snow Brand Milk Products Co., Ltd., Saitama, 350, Japan
SOURCE: Milchwissenschaft (1996), 51(7), 380-382
CODEN: MILCAD; ISSN: 0026-3788
PUBLISHER: VV-GmbH Volkswirtschaftlicher Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Hydrolysis of whey protein isolate (WPI) soln. prior to heat treatment induced preferential **aggregation** of .beta.-lactoglobulin, accompanied by oxidn. of sulfhydryl to disulfide groups or sulfhydryl-disulfide exchange reactions. The degree of **aggregation** depended on the degree of hydrolysis (DH) and protein concn. of the hydrolyzate on heating. Although .beta.-lactoglobulin could not be completely sepd. from .alpha.-lactalbumin, the optimum conditions to remove .beta.-lactoglobulin from WPI soln. were a protein concn. of 5% and a DH of 3.0-5.4% prior to heat treatment.

L3 ANSWER 3 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:760328 HCAPLUS
DOCUMENT NUMBER: 128:31805
TITLE: Lysozyme **aggregation** studied by light scattering. II. variations of **protein concentration**
AUTHOR(S): Georgalis, Yannis; Umbach, Patrick; Raptis, Jannis; Saenger, Wolfram
CORPORATE SOURCE: Inst. Kristallographie, Freie Univ. Berlin, Germany
SOURCE: Acta Crystallographica, Section D: Biological Crystallography (1997), D53(6), 703-712
CODEN: ABCRE6; ISSN: 0907-4449
PUBLISHER: Munksgaard International Publishers Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Static and dynamic light scattering were employed to investigate the behavior of nucleating lysozyme solns. in the range of 0.34-3.08 mM. Preselected concns. of NaCl and (NH4)2SO4 were used to screen the repulsive Coulombic interactions and to trigger **aggregation**. Initially, mass-fractals undergoing diffusion limited-like **aggregation** coexist with monomers or small lysozyme oligomers. The growth kinetics of the fractals delivered observables that exhibited distinct tendencies when examd. as a function of lysozyme concn. The behavior of the observables changed drastically around 2.0 mM lysozyme. Static light scattering expts. revealed progressive restructuring or growth of compact structures at later stages of the **aggregation**. Based on the correlations between the observables an attempt was made to predict whether the examd. solns. would crystallize or not. A tentative scheme, involving the most prominent structures obsd. in nucleating lysozyme solns., was discussed.
REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 4 OF 646 MEDLINE on STN
ACCESSION NUMBER: 2002293187 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12033388
TITLE: Inverse relationship of **protein concentration** and **aggregation**.
AUTHOR: Treuheit Michael J; Kosky Andrew A; Brems David N
CORPORATE SOURCE: Department of Pharmaceutics, Amgen Inc, Thousand Oaks, California 91320, USA.
SOURCE: Pharmaceutical research, (2002 Apr) 19 (4) 511-6.
Journal code: 8406521. ISSN: 0724-8741.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20020530
Last Updated on STN: 20021217
Entered Medline: 20021209

AB PURPOSE: To determine the effect of **protein concentration** on **aggregation** induced through quiescent shelf-life incubation or shipping-related agitation. METHODS: All **aggregation** was measured by size-exclusion high-performance liquid chromatography. **Aggregation** was induced by time-dependent incubation under stationary conditions or by agitation caused by shaking, vortexing, or vibration using simulated shipping conditions. RESULTS: Protein **aggregation** is commonly a second- or higher-order process that is expected to increase with higher **protein concentration**. As expected, for three proteins (PEG-GCSF, PEG-MGDF, and OPG-Fc) that were examined, the **aggregation** increased with higher **protein concentration** if incubated in a quiescent shelf-life setting. However, **aggregation** decreased with higher **protein concentration** if induced by an air/water interface as a result of agitation. This unexpected result may be explained by the rate-limiting effect on **aggregation** of the air/water interface and the critical nature of the air/ water interface to protein ratio that is greatest with decreased **protein concentration**. The non-ionic detergent polysorbate 20 enhanced the **aggregation** observed in the quiescently incubated sample but abrogated the **aggregation** induced by the air/water interface. CONCLUSIONS: The effect of **protein concentration** was opposite for **aggregation** that resulted from quiescent shelf-life treatment compared to induction by agitation. For motionless shelf-life incubation, increased concentration of protein resulted in more **aggregation**. However, exposure to agitation resulted in more **aggregation** with decreased **protein concentration** . These results highlight an unexpected complexity of protein **aggregation** reactions.

L3 ANSWER 5 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1969:499878 HCAPLUS
DOCUMENT NUMBER: 71:99878
TITLE: Correlation between amount of aggregates formed on freezing of immunoglobulin G and **protein concentration**
AUTHOR(S): Hansson, Ulla Britt
CORPORATE SOURCE: Malmo Gen. Hosp., Malmo, Swed.
SOURCE: Acta Chemica Scandinavica (1947-1973) (1969), 23(5), 1828-9
CODEN: ACSAA4; ISSN: 0001-5393
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Serum samples (150 ml. from 1 normal person and 150 ml. from pooled serum) were pptd. with (NH4)2SO4. The single unpooled sample was then purified on DEAE-Sephadex A-50 and dialyzed against 0.05M phosphate buffer. The

pooled sample was purified on DEAE-cellulose and Sephadex G-200 and dissolved in 0.05M phosphate buffer. The purified samples were concd. to 11 g. of globulin/100 ml. and a diln. series of 0.5-11 g./100ml. prepd. The samples were stored 5 months at -20.degree., dild. to 1 g./100 ml. (except the greatest diln.) and centrifuged. In the single-serum sample, 14 aggregated at the 0.5-g./100 ml. diln., and the amt. of aggregate decreased with increasing serum concn. and could no longer be detected at 5 g./100 ml. In the pooled serum sample, 27% aggregated at 0.5 g./100 ml. and 3% at 11 g./100 ml.

L3 ANSWER 6 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:670524 HCAPLUS
DOCUMENT NUMBER: 134:41321
TITLE: Heat-induced **aggregation** of .beta.-lactoglobulin AB at pH 2.5 as influenced by ionic strength and **protein concentration**
AUTHOR(S): Schokker, E. P.; Singh, H.; Pinder, D. N.; Creamer, L. K.
CORPORATE SOURCE: Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, N. Z.
SOURCE: International Dairy Journal (2000), 10(4), 233-240
CODEN: IDAJE6; ISSN: 0958-6946
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Heat-induced (80.degree.) **aggregation** of .beta.-lactoglobulin AB at pH 2.5 was studied using size-exclusion chromatog. in combination with multi-angle laser light scattering, dynamic light scattering and electrophoretic techniques. Upon heating, large aggregates with molar masses of 106-107 Da were formed, whereas the concn. of intermediate-sized aggregates was very low. The rate of disappearance of native-like .beta.-lactoglobulin increased with increasing protein concn. (reaction order 2) and ionic strength. Aggregate size increased slightly with heating time and ionic strength, but was independent of protein concn. Aggregates were held together entirely with non-covalent bonding.
REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 7 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1985:111624 HCAPLUS
DOCUMENT NUMBER: 102:111624
TITLE: Thermal denaturation and **aggregation** of .beta.-lactoglobulin at pH 2.5. Effect of ionic strength and **protein concentration**
AUTHOR(S): Harwalkar, V. R.; Kalab, Miloslav
CORPORATE SOURCE: Food Res. Inst., Agric. Canada, Ottawa, ON, KIA 0C6, Can.
SOURCE: Milchwissenschaft (1985), 40(1), 31-4
CODEN: MILCAD; ISSN: 0026-3788
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Following heat treatment (90%), .beta.-lactoglobulin solns. at pH 2.5 were denatured in an ionic-strength-dependent biphasic manner. In the presence of 0.2M NaCl, the initiated denaturation rate of a 1% .beta.-lactoglobulin soln. was high and subsequently was followed by a lower rate. NaCl at 0.05M and 0.1M had a protective effect on .beta.-lactoglobulin. The same solns. became turbid at ionic strength 0.15 and .beta.-lactoglobulin was pptd. at ionic strength 0.20-0.35. Pptn. was accelerated at ionic strength 0.4-0.5. Similar data were obtained with .beta.-lactoglobulin concns. of 0.25-9.00% heated to 90.degree. for 30 min at pH 2.5 and varying ionic strength. Generally, at a given ionic strength, higher protein concns. resulted in increased rates and extents of denaturation. The stabilizing effect of ionic strength 0-0.15, which has not been reported with other proteins, may result from increased hydration.

Thermal coagulation is dependent on hydrophobic interactions which are enhanced at higher ionic strengths. Thus a desired whey protein product may be obtained by thermal coagulation by adjusting the protein concn. and ionic strength.

L3 ANSWER 8 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:199976 HCAPLUS
TITLE: Mesoscopic simulation of protein **aggregation** as a competitive process to refolding at finite **protein concentration**
AUTHOR(S): Bratko, Dusan; Blanch, Harvey W.
CORPORATE SOURCE: Chemical Engineering, University of California at Berkeley, Berkeley, CA, 94720-1462, USA
SOURCE: Abstracts of Papers - American Chemical Society (2001), 221st, COMP-186
CODEN: ACSRAL; ISSN: 0065-7727
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal; Meeting Abstract
LANGUAGE: English
AB **Aggregation** of protein mols. resulting in the loss of biol. activity and the formation of insol. deposits is a serious problem for the biotechnol. and pharmaceutical industries and in medicine. Considerable exptl. and theor. efforts are being made in order to improve our understanding of, and ability to control the process. We describe a three-dimensional lattice simulation for a multi-chain system of coarse-grained model proteins akin to models developed for studies of protein folding. The model is designed to examine the competition between intramol. interactions leading to the native protein structure, and intermol. assocn. resulting in the formation of aggregates of misfolded chains. For the particular model we apply, the global free energy min. of a pair of protein mols. corresponds to a dimer of native proteins. When three or more mols. interact, clusters of misfolded chains can be more stable than aggregates of native folds. A considerable fraction of native structure, however, is preserved in these cases. Activation barriers sepg. stable domains on the free energy landscape rapidly increase with the size of the protein cluster, hence either the native form or the aggregates can persist in metastable states even if conditions such as temp. or concn. favor a transition to an alternative form. Stability of ordered aggregates increases with the proportion of the sheet-like secondary structure. Refolding yield can be affected by the presence of an addnl. polymer species mimicking the function of a mol. chaperone.

L3 ANSWER 9 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:70107 HCAPLUS
DOCUMENT NUMBER: 133:3863
TITLE: Effect of ascorbic acid and **protein concentration** on the molecular weight profile of bovine serum albumin and .beta.-lactoglobulin by .gamma.-irradiation
AUTHOR(S): Cho, Yongsik; Yang, J. S.; Song, Kyung Bin
CORPORATE SOURCE: Korea Atomic Energy Research Institute, Taejon, 305-600, S. Korea
SOURCE: Food Research International (1999), 32(7), 515-519
CODEN: FORIEU; ISSN: 0963-9969
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effects of ascorbic acid and protein concn. on the mol. wt. size distribution of BSA and .beta.-lactoglobulin were examd. after irradn. of proteins at various doses. Gamma-irradn. of protein solns. caused disruption of the ordered structure of protein mols. resulting in degrdn., crosslinking, and **aggregation** of the polypeptide chains. SDS-PAGE and gel permeation chromatog. study showed that ascorbic acid protected the **aggregation** and degrdn. of proteins by scavenging oxygen radicals produced by irradn. and the effect of irradn. on protein

conformation was more significant at lower concns. of proteins.
REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 10 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:339884 HCAPLUS
DOCUMENT NUMBER: 129:106164
TITLE: Determination of **protein concentration** by enhancement of the preresonance light-scattering of .alpha.,.beta.,.gamma.,.delta.-tetrakis(5-sulfothienyl)porphine
AUTHOR(S): Cheng, Zhi Huang
CORPORATE SOURCE: Institute of Environmental Chemistry, Laboratories of Supramolecular Chemistry, Southwest Normal University, Chungking, 400715, Peop. Rep. China
SOURCE: Analyst (Cambridge, United Kingdom) (1998), 123(6), 1401-1406
CODEN: ANALAO; ISSN: 0003-2654
PUBLISHER: Royal Society of Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A method of protein detn. with the limit of detn. at nanogram levels is proposed by using a common spectrofluorometer to detect the intensity of preresonance light-scattering (PRLS). In the pH range 1.81-4.10, the interactions of .alpha.,.beta.,.gamma.,.delta.-tetrakis(5- sulfothienyl)-porphine, T(5-ST)P, with proteins were studied. It was found that the interactions result in a strongly enhanced preresonance light-scattering signal at 472.0 nm. Mechanism studies showed that the enhanced preresonance light-scattering stems from the J-**aggregation** of T(5-ST)P in the presence of proteins. It was found that the J-**aggregation** process is speedy and is scarcely affected by temp., which supplies a precise method for the detn. of proteins. Different proteins in the range 0-7 .mu.g ml-1 can be detd. with the limits of detn. below 100 ng ml-1 depending on the concn. of T(5-ST)P. The results of detn. for synthetic samples were in agreement with the desired values, and the ones for human serum samples were identical to those obtained according to the Bradford method using CBB G-250.
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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=> d l3 11-20 ibib ab

L3 ANSWER 11 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1979:550978 HCAPLUS
DOCUMENT NUMBER: 91:150978
TITLE: Effect of **protein concentration** on the binding of drugs to human serum albumin. I. Sulfadiazine, salicylate and phenylbutazone
AUTHOR(S): Boobis, Susan W.; Chignell, Colin F.
CORPORATE SOURCE: Pulm. Branch, Natl. Heart, Lung Blood Inst., Bethesda, MD, USA
SOURCE: Biochemical Pharmacology (1979), 28(6), 751-6
CODEN: BCPCA6; ISSN: 0006-2952
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The binding of phenylbutazone [50-33-9], salicylate [69-72-7], or sulfadiazine [68-35-9] (all 250 .mu.g/mL) to albumin decreased with increasing protein concn. in human plasma (undild. or dild. 1:5, 1:20, or 1:100 with 0.1M Na phosphate buffer, pH 7.4) and human serum (0.1 or 1% in 0.1M Na phosphate buffer, pH 7.4). Fluorescence depolarization measurements of dansylglycine complexes with human serum albumin did not reveal "mol. **aggregation**" at high concns., and competition from endogenous ligands, e.g. fatty acids, was not indicated. Binding parameters obtained with dil. albumin solns. when extrapolated to physiol. albumin concns. possibly predicted a higher degree of binding than that obsd. by direct measurement.

L3 ANSWER 12 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1980:162282 HCAPLUS
DOCUMENT NUMBER: 92:162282
TITLE: Influence of milk **protein concentration** on the gelling activity of chymosin and bovine pepsin
AUTHOR(S): Garnot, Pascaline; Corre, Christian
CORPORATE SOURCE: Lab. Rech. Technol., Inst. Natl. Rech. Agron., Rennes, 35042, Fr.
SOURCE: Journal of Dairy Research (1980), 47(1), 103-11
CODEN: JDRSAN; ISSN: 0022-0299
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In both stages of the gelling of milk by the rennet enzymes chymosin [9001-98-3] and pepsin [9001-75-6], using milk retentates, enzymic velocity vs. milk protein concn. (30-175 g/kg) described a std. hyperbola. According to the exptl. conditions either the quasilinear part of the hyperbola was obsd., or the velocity hardly increased and tended to a limiting value. Gelling occurred with a lower degree of proteolysis of .kappa.-casein when the protein concn. increased but a min. amt. of proteolysis (1% of total nitrogenous matter) was necessary for **aggregation** to occur. Gelling time varied with protein concn., pH, and enzyme concn. The final degree of proteolysis of .kappa.-casein was the same whatever the substrate concn. used.

L3 ANSWER 13 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:675758 HCAPLUS
DOCUMENT NUMBER: 132:2885
TITLE: Emulsification properties of whey proteins in their natural environment: effect of whey **protein concentration** at 4 and 18% milk fat
AUTHOR(S): McCrae, C. H.; Law, A. J. R.; Leaver, J.
CORPORATE SOURCE: Hannah Research Institute, Ayr, KA6 5HL, UK
SOURCE: Food Hydrocolloids (1999), 13(5), 389-399
CODEN: FOHYES; ISSN: 0268-005X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The emulsification properties of an isolate of .beta.-lactoglobulin (.beta.-LGI) and a whey protein fraction (WPF) of reconstituted skim milk were studied in a milk-based environment contg. anhyd. milk fat at 4 and 18% by modifying protein concn. At a similar protein-to-fat ratio, fat content per se was not assocd. with changes in initial particle size, but was related to stability during storage. In the more concd. emulsions, creaming was inhibited and no longer affected time-dependent **aggregation** of fat globules, except under the most extreme conditions (i.e. in the presence of a higher proportion of .alpha.-lactalbumin, at high initial particle size and at high storage temp.). However, **aggregation** was enhanced by the presence of a higher proportion of .alpha.-lactalbumin, which was detrimental to phys. stability. Using laser desorption mass spectroscopy, the mol. mass of a significant proportion of WPFs whey protein was obsd. to be increased, possibly as a result of lactolation. In addn., .beta.-LGI contained some

calcium, which slightly increased the concn. of calcium in emulsions made with .beta.-LGI. These differences in mol. mass and calcium concn. did not enhance the stability of emulsions prepd. with .beta.-LGI over that of those prepd. with WPF.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 14 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1992:590414 HCAPLUS
DOCUMENT NUMBER: 117:190414
TITLE: Effect of physical and chemical factors on rheological behavior of commercial soy protein isolates: **protein concentration**, water imbibing capacity, salt addition, and thermal treatment
AUTHOR(S): Wagner, Jorge R.; Sorgentini, Delia A.; Anon, Maria C.
CORPORATE SOURCE: Fac. Cienc. Exactas, UNLP, La Plata, 1900, Argent.
SOURCE: Journal of Agricultural and Food Chemistry (1992), 40(10), 1930-7
CODEN: JAFCAU; ISSN: 0021-8561
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The influence of different factors on the viscosity and rheol. behavior of com. soybean protein isolates was studied. Water-imbibing capacity and protein concn. were interdependent parameters that defined the viscosity. Groups of isolates detd. by differences in the degree of denaturation and **aggregation** due to processing treatments, as reflected in viscosity and different pseudoplastic behaviors, had a significant correlation with those groups based on functional and structural properties or their response to NaCl or Na2SO3 addn. The decrease in viscosity due to salt addn. was highest in denatured samples with a low Ca content. Thermal treatments lead to more viscous dispersions of isolates with partially or even totally denatured proteins. The increase in viscosity is obsd. even in dispersions with viscosity previously decreased by salt addn.

L3 ANSWER 15 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:276476 HCAPLUS
DOCUMENT NUMBER: 124:341360
TITLE: Rennet coagulation of ultrafiltered milk as influenced by **protein concentration**, heat treatment and soluble salts.
AUTHOR(S): Sachdeva, S.; Patel, R. S.; Reuter, H.
CORPORATE SOURCE: Division Dairy Technology, National Dairy Research Institute, Kamal, India
SOURCE: Indian Journal of Dairy Science (1995), 48(1), 57-62
CODEN: IJDSAI; ISSN: 0019-5146
PUBLISHER: Indian Dairy Association
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The study compared the rate of coagulation and the firmness of curd of retentates obtained from pasteurized and severely heated milks and the effects of addn. of salts. The rennet coagulation time (RCT) decreased and the curd firmness increased with the increase in protein concn. of the ultrafiltered (UF) milk. Severely heated milk (95.degree.C/5 min) when ultrafiltered to a four fold concn. showed higher RCT than pasteurized milk retentate. The curd firmness of the renneted UF retentate from severely heated milk was comparable to that of renneted pasteurized milk. The RCT increased on addn. of sodium chloride to the retentate while the curd firmness decreased but addn. of calcium chloride caused the reverse effect. Severe heat treatment and addn. of sodium chloride might aid the control of curd firmness in the manuf. of semi-hard and hard varieties of cheese from UF retentates.

L3 ANSWER 16 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1998:66519 HCAPLUS
DOCUMENT NUMBER: 128:101305
TITLE: Gel Formation from Industrial Milk Whey Proteins under Hydrostatic Pressure: Effect of Hydrostatic Pressure and **Protein Concentration**
AUTHOR(S): Kanno, Choemon; Mu, Tai-Hau; Hagiwara, Toshio; Ametani, Michiko; Azuma, Norihiro
CORPORATE SOURCE: Department of Applied Biochemistry, Utsunomiya University, Utsunomiya, 321, Japan
SOURCE: Journal of Agricultural and Food Chemistry (1998), 46(2), 417-424
CODEN: JAFCAU; ISSN: 0021-8561
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effects of high hydrostatic pressure and protein concn. on the denaturation and gelation of whey protein were investigated. Industrial whey protein isolate (WPI) and whey protein conc. (WPC) solns. (pH 6.8) at various concns. were pressurized for 10 min at 30.degree.C under 200-1000 MPa. With the WPI soln., the concn. for affecting the turbidity was 1% and was 6% for the viscosity at 400 MPa, while for inducing gelation, it was 10% at 600 MPa. With the WPC soln., the viscosity changed at a concn. >12%, and gel formation began at >18% at 400 MPa. The hardness and breaking stress of pressure-induced WPI gels increased with increasing concn. of WPI (12-18%) and hydrostatic pressure, the ratings for the 20% WPC gels being one-third those of the 20% WPI gels. The soly. of proteins from the pressure-induced WPI gels decreased with increasing pressure, while that of WPC gel induced at >600 MPa remained const. at about .apprx.50%. The microstructure of the WPI gels had a porous network form, whereas the WPC gels were irregular particulates. .beta.-Lactoglobulin, .alpha.-lactalbumin, and serum albumin preferentially participated in pressure-induced **aggregation** and gelation through S-S bonding.
REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 17 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:255552 HCAPLUS
DOCUMENT NUMBER: 136:397559
TITLE: Microcalorimetric studies of insulin and Zn(II)-insulin over a wide range of pH and **protein concentration**
AUTHOR(S): Khachidze, D. G.; Kalandadze, Y. L.; Monaselidze, J. R.
CORPORATE SOURCE: Institute of Physics, Georgian Academy of Sciences, Tbilisi, 380077, Gabon
SOURCE: Journal of Biological Physics and Chemistry (2001), 1(2), 64-67
CODEN: JBPCAJ; ISSN: 1512-0856
PUBLISHER: Collegium Basilea
DOCUMENT TYPE: Journal
LANGUAGE: English
AB It is shown that the denaturation process of a medicinal prepn. of insulin in dil. soln. covers the temp. range 50-96.degree.. The excess heat capacity (.DELTA.Cp) increases linearly with temp. and is equal to 0.29 J g-1 K-1 at 62.degree.. A significant peak with Td = 81.5.degree., .DELTA.Hd = 43.4 J/g and .DELTA.Td = 13.5.degree. is obsd. at 62-96.degree.. Calcn. of the ratio .DELTA.Hdcal/.DELTA.Heff of calorimetric to effective (van't Hoff) enthalpies gives the value 0.83 which is fairly close to 1.0, indicating that the denaturation process of insulin proceeds as usually obsd. for compact globular proteins. The melting process of a medicinal prepn. of Zn(II)-insulin, which is not an optically transparent soln., has three stages with Td1 = 77.0, TD2 = 82.5 and TD3 = 91.5.degree.; all these stages correspond to various **aggregation** states of the protein. Hence it is inferred that Zn(II)-insulin exists in three different energetic states depending on

concn.: in the concn. range 0.1-0.5%, .DELTA.Hd decreases from 25.3 to 16.0 J/g; in the concn. range 1.5-40% is characterized by a const. value of .DELTA.Hd equal to 16.0 J/g; in the concn. range 40-80%, .DELTA.Hd drops monotonously from 16.0 to 5.2 J/g. It is evinced that this decrease of .DELTA.Hd is connected with disruption of the protein hydration shell.
REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 18 OF 646 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 97:47423 SCISEARCH
THE GENUINE ARTICLE: WA881
TITLE: Protein **aggregation** and adsorption upon in vitro refolding of recombinant Pseudomonas lipase
AUTHOR: Lee Y P (Reprint); Rhee J S
CORPORATE SOURCE: KOREA ADV INST SCI & TECHNOL, DEPT BIOL SCI, TAEJON 305701, SOUTH KOREA; LG CHEM LTD, BIOTECH RES INST, TAEJON 305380, SOUTH KOREA
COUNTRY OF AUTHOR: SOUTH KOREA
SOURCE: JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, (DEC 1996) Vol. 6, No. 6, pp. 456-460.
Publisher: KOREAN SOC APPLIED MICROBIOLOGY, 635-4 YEOGSAM-DONG, KANGNAM-KU, SEOUL, 135-703, KOREA.
ISSN: 1017-7825.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 12
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Recombinant Pseudomonas lipase was used to study protein **aggregation** and adsorption upon in vitro refolding. Protein adsorption as well as **aggregation** was responsible for major side reactions upon in vitro refolding as a function of **protein concentration**. The optimal range of **protein concentration** was determined by the relative contribution of protein **aggregation** and adsorption. Above the optimal range, the yield of active lipase inversely correlated with protein **aggregation**, showing a competition between folding and **aggregation**. However, adsorption of protein rather than protein **aggregation** is thought to contribute as a major side reaction of the refolding process at sub-optimal concentrations at which the formation of aggregates should be more reduced. Protein **aggregation** was influenced by the amount of guanidine hydrochloride in the refolding solvent. The refolding temperature was a critical factor determining the extent of protein **aggregation**. The refolding yield was also affected by the dilution fold and dilution mode, which suggests that the refolding process might kinetically compete with the rate of mixing.

L3 ANSWER 19 OF 646 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1991:77665 HCAPLUS
DOCUMENT NUMBER: 114:77665
TITLE: Study of thermotropic globular protein gels by differential scanning calorimetry. II. The effect of ionic strength and **protein concentration** on lysozyme gelation for different gels
AUTHOR(S): Sochava, I. V.; Belopol'skaya, T. V.; Kazitsyna, S. Yu.
CORPORATE SOURCE: Inst. Phys., Leningrad State Univ., Leningrad, USSR
SOURCE: Biofizika (1990), 35(5), 756-61
CODEN: BIOFAI; ISSN: 0006-3029
DOCUMENT TYPE: Journal
LANGUAGE: Russian
AB The effect of ionic strength and protein concn. on the behavior of lysozyme solns. with heating is studied by calorimetry. Solns. under study have pH in the range 1.7-9.0. By the control of **aggregation** with changing concns. of NaCl and protein,the essential extension of pH

range could be achieved in which a thermoreversible gel is formed. The structure of the branched gel could be changed by the above mentioned controlling factors such that the gel becomes melting.

L3 ANSWER 20 OF 646 MEDLINE on STN
ACCESSION NUMBER: 83127243 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6897615
TITLE: Calcium-promoted resonance energy transfer between fluorescently labeled proteins during **aggregation** of chromaffin granule membranes.
AUTHOR: Morris S J; Sudhof T C; Haynes D H
CONTRACT NUMBER: GM23990 (NIGMS)
SOURCE: Biochimica et biophysica acta, (1982 Dec 22) 693 (2) 425-36.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198304
ENTRY DATE: Entered STN: 19900318
Last Updated on STN: 19970203
Entered Medline: 19830407

AB Proteins of the chromaffin granule membrane were covalently labeled in situ with sulfhydryl-specific fluorophores. Using MIANS (maleimide iodoaminonaphthyl sulfonate) as the donor and fluorescein mercury acetate or fluorescein-5-maleimide as the acceptor. Forster fluorescence resonance energy transfer (FRET) could be employed to measure the degree of inter-membrane and intra-membrane protein-protein contact upon Ca2+-induced **aggregation** of the membranes. The four major findings were: (1) Raising the Ca2+ concentration to approx. 500 microM causes the proteins to aggregate in the plane of the membrane. This is demonstrated by Ca2+-induced increases in the fluorescence resonance energy transfer in double labeled membranes. This effect is not **protein-concentration** dependent and occurs at calcium concentrations too low for granule **aggregation**, implying intra-membrane protein clustering or patching. To our knowledge this is the first direct demonstration of the fluid mosaic nature of subcellular organelles. (2) If two sets of granules are labeled separately, Ca2+-induced **aggregation** brings at least 74% of the labeled proteins into close transmembrane proximity. This effect is also observed at 10-100-fold slower rates in the absence of calcium and can be greatly reduced by depleting the granule membrane of labeled peripheral proteins. It is enhanced if the granules are aggregated by Ca2+ or K+. We conclude that (some) peripheral proteins can transfer from one membrane surface to another. (3) **Aggregation** of separately labeled sets of membranes by Ca2+ also produces transmembrane energy transfer since: (a) the Km for Ca2+-induced quantum transfer is in the same range as the Km for **aggregation**; (b) the reaction is **protein-concentration** dependent; (c) reversal of **aggregation** also (partially) reverses donor quenching. (4) A kinetic analysis of the transmembrane effect shows it to be 5-10-fold slower than **aggregation** itself, supporting earlier suggestions (Haynes, D.H., Kolber, M. and Morris, S.J., (1979) J. Theor. Biol. 81, 713-743) that lipid and protein rearrangements are secondary to granule membrane **aggregation**.

=> d his

(FILE 'HOME' ENTERED AT 14:57:07 ON 26 JUL 2004)

FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT 14:58:08 ON 26 JUL 2004
L1 1416 S PROTEIN CONCENTRATION AND AGGREGATION

L2 646 DUP REM L1 (770 DUPLICATES REMOVED)
L3 646 FOCUS L2 1-

=> d l3 21-30 ibib ab

L3 ANSWER 21 OF 646 MEDLINE on STN
ACCESSION NUMBER: 2002293634 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11979521
TITLE: A metal binding in the polypeptide chain improves the folding efficiency of a denatured and reduced protein.
AUTHOR: Ohkuri Takatoshi; Ueda Tadashi; Yoshida Yuichiro; Abe Yoshito; Hamasaki Naotaka; Imoto Taiji
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan.
SOURCE: Biopolymers, (2002 Jul 5) 64 (2) 106-14.
Journal code: 0372525. ISSN: 0006-3525.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020530
Last Updated on STN: 20020703
Entered Medline: 20020702

AB In order to examine the effect of a metal binding to the polypeptide chain on the **aggregation** of a protein in the refolding process, we prepared a mutant hen lysozyme possessing the same Ca(2+) binding site as in human alpha-lactalbumin by Escherichia coli expression system (Ser(-1) CaB lysozyme). In the presence of 2 mM CaCl(2), the refolding yield of Ser(-1) CaB lysozyme at a low **protein concentration** (25 microg/mL) was similar to that of the wild-type lysozyme (80%), but that at high **protein concentration** (200 microg/mL) decreased (15%) due to **aggregation** comparing to that of the wild-type lysozyme (45%). However, the refolding yield of Ser(-1) CaB lysozyme in the presence of 100 mM CaCl(2) even at a **protein concentration** of 200 microg/mL was 80% and was higher than that of the wild-type lysozyme. From analysis of chemical shift changes of the cross peaks in the backbone region of total correlated spectroscopy (TOCSY) spectra of a decapeptide possessing the same calcium binding site as in Ser(-1) CaB lysozyme in the presence of various concentrations of Ca(2+), it was suggested that the dissociation constant of Ca(2+)-peptide complex was estimated to be 20-36 mM. Moreover, the solubility of the denatured Ser(-1) CaB lysozyme in the presence of 100 mM CaCl(2) was higher than that in the presence of 2 mM CaCl(2) whereas the solubility of the denatured Ser(-1) lysozyme in the presence of 100 mM CaCl(2) was not higher than that in the presence of 2 mM CaCl(2). Therefore, it was concluded that the reduced lysozyme possessing the Ca(2+) binding site was efficiently folded in the presence of high concentration of Ca(2+) (100 mM) even at high **protein concentration** due to depression of **aggregation** by the binding of Ca(2+) to the polypeptide chain in Ser(-1) CaB lysozyme.

L3 ANSWER 22 OF 646 MEDLINE on STN
ACCESSION NUMBER: 83234447 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6861743
TITLE: Ca2+ and Mg2+-dependent complex formation of tropomyosin with phosphotroponin (P1TI2C) or dephosphotroponin (TI2C).
AUTHOR: Jahnke U; Heilmeyer L M Jr
SOURCE: European journal of biochemistry / FEBS, (1983 Jul 1) 133 (3) 591-7.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 198308
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19830811

AB The reduced viscosity of troponin and dephosphotroponin is independent of the **protein concentration** in both states, either metal-free or with troponin C saturated with Ca2+ or Mg2+; that of tropomyosin increases linearly as function of the **protein concentration**, indicating **aggregation**. Addition of troponin to tropomyosin increases the reduced viscosity over the expected value being maximal at a 1:1 molar ratio of both proteins. The reduced viscosity of a 1:1 molar mixture of phosphotroponin-Mg4 or dephosphotroponin-Mg3 increases in two phases as function of the total **protein concentration**, indicating the formation of two kinds of troponin-tropomyosin complexes. In the first phase, troponin and tropomyosin form a non-aggregating 1:1 complex, which is characterized by a value of 0.45 dl/g for the intrinsic viscosity and a sedimentation coefficient of 3.6 S. Employing these two values a molecular weight of 150 000 can be calculated, which is in the range of the sum of molecular weights for troponin and tropomyosin (156 000). In the second phase the troponin-tropomyosin complex aggregates further, a process described by:n (troponin-tropomyosin) leads to (troponin-tropomyosin)n. This further **aggregation** occurs upon saturation of the Ca2+-specific sites in troponin C. A model is discussed which explains the shortening of 1.5 nm per tropomyosin molecule upon the shift of tropomyosin from the periphery into the groove of the actin filament by tropomyosin **aggregation**
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L3 ANSWER 23 OF 646 MEDLINE on STN
ACCESSION NUMBER: 1999081557 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9865959
TITLE: Effect of denaturant and protein concentrations upon protein refolding and **aggregation**: a simple lattice model.
AUTHOR: Gupta P; Hall C K; Voegler A C
CORPORATE SOURCE: Department of Chemical Engineering, North Carolina State University, Raleigh 27695, USA.
SOURCE: Protein science : a publication of the Protein Society, (1998 Dec) 7 (12) 2642-52.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990316
Last Updated on STN: 19990316
Entered Medline: 19990303

AB We present a study of the competition between protein refolding and **aggregation** for simple lattice model proteins. The effect of solvent conditions (i.e., the denaturant concentration and the **protein concentration**) on the folding and **aggregation** behavior of a system of simple, two-dimensional lattice protein molecules has been investigated via (dynamic Monte Carlo simulations. The population profiles and **aggregation** propensities of the nine most populated intermediate configurations exhibit a complex dependence on the solution conditions that can be understood by considering the competition between intra- and interchain interactions. Some of these configurations are not even seen in isolated chain simulations; they are observed to be highly **aggregation** prone and are stabilized primarily by the **aggregation** reaction in multiple-chain systems. **Aggregation** arises from the association of partially folded intermediates rather than from the association of denatured random-coil states. The **aggregation**

reaction dominates over the folding reaction at high **protein concentration** and low denaturant concentration, resulting in low refolding yields at those conditions. However, optimum folding conditions exist at which the refolding yield is a maximum, in agreement with some experimental observations.

L3 ANSWER 24 OF 646 MEDLINE on STN
ACCESSION NUMBER: 2004076269 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 14966801
TITLE: Optimized procedure for renaturation of recombinant human bone morphogenetic protein-2 at high **protein concentration**.
AUTHOR: Vallejo Luis Felipe; Rinas Ursula
CORPORATE SOURCE: Biochemical Engineering Division, GBF German Research Center for Biotechnology, Mascheroder Weg 1, 38124 Braunschweig, Germany.
SOURCE: Biotechnology and bioengineering, (2004 Mar 20) 85 (6) 601-9.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20040218
Last Updated on STN: 20040305

AB The human gene encoding the mature form of bone morphogenetic protein-2 (hBMP-2), a dimeric disulfide-bonded protein of the cystine knot growth factor family, was expressed in recombinant Escherichia coli using a temperature-inducible expression system. The recombinant protein was produced in the form of cytoplasmic inclusion bodies and the effect of different variables on the renaturation of rhBMP-2 was investigated. In particular, variables such as pH, redox conditions, **protein concentration**, temperature, the presence of different types of **aggregation** suppressors, and host cell contaminants were studied with respect to their effect on **aggregation** during refolding and on the final renaturation yield of rhBMP-2. It is shown that the renaturation yield is particularly sensitive to pH, temperature, **protein concentration**, and the presence of **aggregation** suppressors. In contrast, little effect of the redox conditions and the ionic strength on the renaturation yield was observed, as equal yields were obtained in a broad range of reduced to oxidized glutathione ratios and concentrations of NaCl, respectively. The **aggregation** suppressor 2-(cyclohexylamino)ethanesulfonic acid (CHES) proved to be superior with respect to the final renaturation yield, although, in comparison to the more common arginine, it was less efficient in preventing **aggregation** of rhBMP-2 during refolding. Detergent washing of inclusion bodies was sufficient, as further purification of rhBMP-2 prior to refolding was without effect on the final renaturation yield. An increase in the concentration of renatured rhBMP-2 was achieved by a pulsed refolding procedure by which up to a total amount of 2.1 mg mL(-1) rhBMP-2 could be transferred in seven pulses into the renaturation buffer with an overall refolding yield of 38%, corresponding to 0.8 mg mL(-1) renatured dimeric rhBMP-2. Furthermore, a simplified purification procedure is presented that also includes freeze-drying for long-term storage of biologically active rhBMP-2. Finally, it is shown that the appearance of rhBMP-2 variants could be avoided by using a host strain overexpressing rare codon tRNAs.

L3 ANSWER 25 OF 646 MEDLINE on STN
ACCESSION NUMBER: 2001663523 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11708788
TITLE: Thermal stability of human ferritin: concentration dependence and enhanced stability of an N-terminal fusion mutant.

AUTHOR: Kim S W; Kim Y H; Lee J
CORPORATE SOURCE: Laboratory of Biomolecular Process Engineering, Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon 305-600, South Korea.
SOURCE: Biochemical and biophysical research communications, (2001 Nov 23) 289 (1) 125-9.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011119
Last Updated on STN: 20020123
Entered Medline: 20011227

AB Though human L-chain ferritin has been known to be more resistant to physical denaturation than H-type ferritin, its stability characteristics and kinetic information have not been reported in detail. Overexpressed recombinant ferritin (FTN) in Escherichia coli formed inclusion bodies through noncovalent molecular interaction and easily dissolved with regaining the iron-uptake activity by a simple pH-shift process at high **protein concentration** (>600 mg l(-1)). FTN was relatively thermostable at low **protein concentration** (0.2 g l(-1)), but it became extremely thermolabile at high **protein concentration** (1.3 g l(-1)), i.e., more than 80% of FTN was coprecipitated within 5 min under the same heat-induced denaturation condition. **Aggregation** rate constant for initial 5 min at high **protein concentration** was 6.04 x 10(-3) s(-1) for FTN. Surprisingly, glucagon. ferritin mutant (GFTN), consisting of an N-terminus fusion partner, human glucagon (29-residue alpha-helical peptide), showed significantly enhanced thermal stability even at high **protein concentration**. That is, in spite of 40-min heat treatment, more than 50% of GFTN the still remained soluble with maintaining the same functional properties. The **aggregation** rate constants were 2.75 x 10(-4) and 2.80 x 10(-4) s(-1) at low and high concentration, respectively, for GFTN. These results suggest a critical participation of the N-terminal domain of ferritin in the temperature-induced **aggregation** pathway. Presumably, partially denatured amino terminus of FTN is involved in nonspecific molecular interaction resulting in the off-pathway **aggregation**. It is notable that the purified GFTN showed the same molar capacity of iron (Fe(+3)) storage as standard ferritin. From the analysis of fluorescence emission spectrum, the physical stability of GFTN was also very comparable to that of standard ferritin under the various denaturation conditions induced by GdnHCl.

L3 ANSWER 26 OF 646 MEDLINE on STN
ACCESSION NUMBER: 1998241383 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9572876
TITLE: Folding of firefly (Photinus pyralis) luciferase: **aggregation** and reactivation of unfolding intermediates.
AUTHOR: Herbst R; Gast K; Seckler R
CORPORATE SOURCE: Universitat Regensburg, Institut fur Biophysik und Physikalische Biochemie, D-93040 Regensburg, Germany.
SOURCE: Biochemistry, (1998 May 5) 37 (18) 6586-97.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 19980618
Last Updated on STN: 19980618

Entered Medline: 19980608

AB The guanidine-induced unfolding of firefly (Photinus pyralis) luciferase involves two inactive equilibrium intermediates and is freely reversible at low **protein concentration** and low temperature. However, reactivation is exceedingly slow so that the equilibrium is attained only after several days of incubation and reactivation yields decrease strongly with increasing **protein concentration** , suggesting that **aggregation** is a competing side reaction [Herbst et al. (1997) J. Biol. Chem. 272, 7099-7105]. We investigated the role of the equilibrium intermediates in the **aggregation** process using size-exclusion chromatography and dynamic light scattering to monitor their association state. Although the more unfolded intermediate aggregated much more rapidly, both intermediates associated irreversibly without a conformational change visible by fluorescence or circular dichroism, forming small oligomers which remained soluble in the presence of the denaturant. The association kinetics are compatible with a nucleated polymerization mechanism. Unfolding kinetics at 1 M denaturant indicated the presence of a further inactive intermediate capable to reactivate rapidly with kinetics similar to those observed for luciferase reactivation in the presence of cell extracts. The data suggest a kinetic trap in luciferase refolding that is accessible from both equilibrium intermediate conformations and is avoided in the presence of molecular chaperones.

L3 ANSWER 27 OF 646 MEDLINE on STN
ACCESSION NUMBER: 2000026757 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10552857
TITLE: Thermal **aggregation** of patatin studied in situ.
AUTHOR: Pots A M; ten Grotenhuis E; Gruppen H; Voragen A G; de Kruif K G
CORPORATE SOURCE: Centre for Protein Technology TNO-WAU, Wageningen, The Netherlands.
SOURCE: Journal of agricultural and food chemistry, (1999 Nov) 47 (11) 4600-5.
Journal code: 0374755. ISSN: 0021-8561.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000131
Last Updated on STN: 20000131
Entered Medline: 20000118

AB In this work dynamic light scattering was used to study the thermal **aggregation** of patatin in situ, to elucidate the physical **aggregation** mechanism of the protein and to be able to relate the **aggregation** behavior to its structural properties. The dependence of the **aggregation** rates on the temperature and the ionic strength suggested a mechanism of slow coagulation, being both diffusion and chemically limited. The **aggregation** rate dependence on the **protein concentration** was in accordance with the mechanism proposed. The **aggregation** rates as obtained at temperatures ranging from 40 to 65 degrees C correlated well with unfolding of the protein at a secondary level. Small-angle neutron scattering and dynamic light scattering results were in good accordance; they revealed that native patatin has a cylindrical shape with a diameter and length of 5 and 9.8 nm, respectively.

L3 ANSWER 28 OF 646 MEDLINE on STN
ACCESSION NUMBER: 96218879 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8648515
TITLE: Effect of link **protein concentration** on articular cartilage proteoglycan **aggregation**.
AUTHOR: Tang L H; Buckwalter J A; Rosenberg L C
CORPORATE SOURCE: Montefiore Medical Center, Bronx, New York, USA.

SOURCE: Journal of orthopaedic research : official publication of the Orthopaedic Research Society, (1996 Mar) 14 (2) 334-9. Journal code: 8404726. ISSN: 0736-0266.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960805
Last Updated on STN: 19990129
Entered Medline: 19960725

AB Previous work has shown that alterations in proteoglycan aggregates are among the first changes detected with aging, disuse, and degeneration of articular cartilage, yet the cause or causes of these alterations remain unknown. To determine if differences in link **protein concentration** can explain alterations in the assembly, size, and stability of articular cartilage proteoglycan aggregates, we isolated proteoglycan monomer (aggrecan) and link protein from adult bovine articular cartilage and then assembled proteoglycan aggregates from aggrecan and 0.8% hyaluronan relative to aggrecan weight, in the presence of 0, 2, 4, 6, 8, 10, 15, and 20% concentrations of link protein relative to aggrecan weight. We determined the amount, sedimentation coefficient, and stability of the aggregates by analytical ultracentrifugation and measured their dimensions by electron microscopy with use of the monolayer technique. Increased aggregate size, as determined by ultracentrifugation, was directly correlated with an increased number of aggrecans per aggregate and with increased hyaluronan length, as determined by electron microscopy. The concentration of link protein significantly influenced **aggregation**: concentrations of 6-8% produced maximum **aggregation**, aggregate stability, and uniformity of aggrecan spacing; concentrations greater than 10% led to the formation of superaggregates (aggregates with sedimentation velocities greater than 100 S that may result from linking two or more hyaluronan filaments) but decreased aggregate stability; and concentrations of less than 4% link protein significantly decreased **aggregation**, the size and stability of aggregates, and the regularity of aggrecan spacing. The latter observations suggest that a decline in the concentration of link protein could decrease the organization and stability of the articular cartilage matrix.

L3 ANSWER 29 OF 646 MEDLINE on STN
ACCESSION NUMBER: 90079138 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2592815
TITLE: The study on the platelet **aggregation** inhibition activity of placental brush border (the mechanism of platelet **aggregation** inhibition).
AUTHOR: Iioka H; Hisanaga H; Moriyama I; Akasaki M; Nabuchi K; Katakami Y; Katoh Y; Ichijo M
CORPORATE SOURCE: Department of Obstetrics and Gynecology, Nara Medical University.
SOURCE: Nippon Sanka Fujinka Gakkai zasshi, (1989 Dec) 41 (12) 1916-20. Journal code: 7505749. ISSN: 0300-9165.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199001
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19900125

AB To clarify the role of placental brush border in the regulation of placental microcirculation, we investigated the platelet **aggregation** inhibition activity of placental brush border membrane vesicles (BBMV) and obtained the following results. 1. There was very

strong platelet **aggregation** inhibiting activity in placental BBMV. In the 20-40 micrograms/ml **protein concentration**, the BBMV almost completely inhibited the platelet **aggregation** induced by ADP, arachidonic acid and collagen, and in the 100-150 micrograms/ml **protein concentration**, the BBMV almost completely inhibited the platelet **aggregation** induced by ristocetin. 2. There existed a very strong ADP degrading activity (ADPase activity) in the placental BBMV. The platelet **aggregation** activity of ADP was completely lost when ADP was preincubated with the placental BBMV. 3. The placental BBMV inhibited platelet malondialdehyde (MDA) production. With the 20 micrograms/ml and 100 micrograms/ml protein concentrations of placental BBMV, 22% and 65% of platelet MDA production was inhibited respectively.

L3 ANSWER 30 OF 646 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:248886 BIOSIS
DOCUMENT NUMBER: PREV199799548089
TITLE: Protein and salt effects on Ca-2+-induced cold gelation of whey protein isolate.
AUTHOR(S): Hongsprabhas, P.; Barbut, S.
CORPORATE SOURCE: Dep. Food Sci., Univ. Guelph, Guelph, ON N1G 2W1, Canada
SOURCE: Journal of Food Science, (1997) Vol. 62, No. 2, pp. 382-385.
CODEN: JFDSAZ. ISSN: 0022-1147.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Jun 1997
Last Updated on STN: 13 Jun 1997

AB Increasing whey **protein concentration** (from 6 to 10% w/v) decreased gel opacity but increased gel strength and water-holding capacity (WHC). Increasing CaCl-2 concentration (from 5 to 150 mM) increased gel opacity and gel strength at the high **protein concentration** (i.e., 10%). However, it lowered gel strength at **protein concentration** lt 10%. Young's modulus and distance to fracture values indicated that gels were most rigid at 30 mM CaCl-2, at which point the extent of **aggregation** (measured by turbidity) was the highest. Increasing CaCl-2 concentration from 5 to 150 mM slightly affected the WHC of Ca-2+-induced gels. **Protein concentration** was the major factor in determining fracture properties and WHC.

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	77.41	77.83

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-12.50	-12.50

STN INTERNATIONAL LOGOFF AT 15:05:51 ON 26 JUL 2004

```
=> s Urate oxidase is a tetramer
L1            0 URATE OXIDASE IS A TETRAMER

=> s Urate oxidase and  tetramer
L2           17 URATE OXIDASE AND TETRAMER

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3            7 DUP REM L2 (10 DUPLICATES REMOVED)

=> focus l3
PROCESSING COMPLETED FOR L3
L4            7 FOCUS L3 1-

=> d l4 1-7 ibib ab

L4   ANSWER 1 OF 7  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         1993:97711  HCAPLUS
DOCUMENT NUMBER:          118:97711
TITLE:                    Kinetic and catalytic characterization of
                          urate oxidase from Chlamydomonas
                          reinhardtii
AUTHOR(S):                Alamillo, Josefa M.; Cardenas, Jacobo; Pineda, Manuel
CORPORATE SOURCE:         Fac. Cienc., Univ. Cordoba, Cordoba, 14071, Spain
SOURCE:                   Journal of Molecular Catalysis (1992), 77(3), 353-64
                          CODEN: JMCADS; ISSN: 0304-5102
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB   Urate oxidase (EC 1.7.3.3) from Chlamydomonas
     reinhardtii is a tetramer of Mr = 124,000, composed of
     similar-sized subunits and contg. 4 atoms of copper per enzyme mol. The
     enzyme exhibits a strict specificity toward its substrates, oxygen and
     urate, and has optimal activity in the pH 8.5-9.5 range, as detd. by the
     Vmax/Km ratio. Purified uricase obeys hyperbolic kinetics for urate but
     shows a sigmoidal response to oxygen (Hill's coeff. = 3.7), indicative of
     a pos. homotropic cooperativity. Under steady-state conditions, sets of
     intersecting lines were found in the double-reciprocal plots, which
     indicates that uricase reaction proceeds through a ternary complex
     involving enzyme, urate and oxygen. Since each substrate bonded to the
     enzyme in the absence of the other, a random mechanism is proposed for the
     complex formation. Uricase activity was competitively inhibited by
     hypoxanthine, xanthine and their 8-aza derivs., as well as by oxonate,
     allantoin and CO2. Hydrogen peroxide acted as an uncompetitive inhibitor,
     and 1- and 9-methylurate showed mixed inhibition. Dissocn. consts. calcd.
     from the effect of pH on Km and Vmax indicate that 2 dissociable groups
     with pK values of about 7.6 and 10.0 are probably involved in both
     substrate binding and catalytic oxidn. Inhibition by the group-specific
     reagents diethylpyrocarbonate and phenylglyoxal strongly suggests that
     those dissociable groups are histidyl and arginyl residues. Apparently,
     copper and dissociable groups on the enzyme interact with C:O and NH
     groups on urate.

L4   ANSWER 2 OF 7     MEDLINE on STN
ACCESSION NUMBER:   2002399993     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 12149119
TITLE:              Modification of a reactive cysteine explains differences
                    between rasburicase and Uricozyme, a natural Aspergillus
                    flavus uricase.
AUTHOR:             Bayol Alain; Capdevielle Joel; Malazzi Pascal; Buzy
                    Armelle; Claude Bonnet Marie; Colloc'h Nathalie; Mornon
                    Jean-Paul; Loyaux Denis; Ferrara Pascual
CORPORATE SOURCE:   Analysis and Quality Control Unit, Sanofi-Synthelabo
                    Recherche, Innopole, Voie no. 1, BP 137, 31676 Labege
                    Cedex, France.. alain.bayol@sanofi-synthelabo.com
SOURCE:             Biotechnology and applied biochemistry, (2002 Aug) 36 (Pt
```

1) 21-31.
Journal code: 8609465. ISSN: 0885-4513.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20020801
Last Updated on STN: 20030128
Entered Medline: 20030127

AB **Urate oxidase** is used in humans for the control of uric acid in patients receiving chemotherapy. Rasburicase (Fasturtec/Elitek), a recombinant **urate oxidase** expressed in Saccharomyces cerevisiae, was compared with Uricozyme, the natural enzyme produced by Aspergillus flavus. Rasburicase has a higher purity as demonstrated by SDS/PAGE and chromatographic analysis and a better specific activity. The differences observed for Uricozyme are likely attributable to the previously used purification process, which modifies the enzyme. The production process of rasburicase, on the other hand, preserves the structure of the molecule. MS analysis shows that Uricozyme contains a cysteine adduct on Cys(103). In the crystal structure, the sulphur atom of the cysteine residue in position 103 is orientated to the external surface of the **tetramer**, whereas the sulphur atom of two other cysteine residues (Cys(35) and Cys(290)) is orientated to the centre of the canal formed by the **tetramer**. The same adduct is produced by simple incubation of the rasburicase with cysteine.

L4 ANSWER 3 OF 7 MEDLINE on STN
ACCESSION NUMBER: 2002186237 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11910495
TITLE: **Urate oxidase** from the rust Puccinia recondita is a heterotetramer with two different-sized monomers.
AUTHOR: Aguilar Miguel; Montalbini Paolo; Pineda Manuel
CORPORATE SOURCE: Departamento de Bioquimica y Biologia Molecular, Universidad de Cordoba, Campus Rabanales, Edificio C-6, 1 Planta, 14071-Cordoba, Spain.
SOURCE: Current microbiology, (2002 Apr) 44 (4) 257-61.
Journal code: 7808448. ISSN: 0343-8651.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020403
Last Updated on STN: 20020517
Entered Medline: 20020516

AB Uricase (urate: oxygen oxidoreductase; EC 1.7.3.3) from the rust Puccinia recondita was purified to electrophoretic homogeneity. Preparations with a specific activity of 8.4 U/mg were used for characterization of the enzyme, which showed a strong similarity to other plant and fungal urate oxidases. The enzyme had a pH optimum of 9.0, a K(m) of 35 microM for urate, and it was inhibited only by oxonate and xanthine. A molecular mass of 152 kDa was estimated for the native protein. SDS-PAGE analysis revealed a striking difference to most urate oxidases, since two different-sized subunits were detected. These results suggest that P. recondita uricase is a **tetramer** with two types of subunits.

L4 ANSWER 4 OF 7 MEDLINE on STN
ACCESSION NUMBER: 79074724 MEDLINE
DOCUMENT NUMBER: PubMed ID: 724662
TITLE: [**Urate oxidase** from pig liver: biochemical and immunological properties].
Uratoksidaza iz pecheni svin'i: nekotorye biokhimicheskie i

immynologicheskie svoistva fermenta.
AUTHOR: Surina T Ia; Cherniavskaia M A; Maksimova A S; Kagan A S; Kachkin A P; Levi E V
SOURCE: Prikladnaia biokhimiia i mikrobiologiia, (1978 Jul-Aug) 14 (4) 533-42.
Journal code: 0023416. ISSN: 0555-1099.
PUB. COUNTRY: USSR
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197902
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19900314
Entered Medline: 19790212

AB A highly purified uratoxidase was isolated from the pig liver. The sedimentation coefficient of the enzyme was 6.96 S and the molecular weight was 122,000 +/- 4,000. The enzyme was a **tetramer** consisting of subunits with a molecular weight of 31,600 +/- 2,500. Uratoxidase showed high substrate specificity with 0.05 M borate buffer, pH 8.5. During competitive inhibition 8-azaxanthine (Ki = 3.1 X 10(-7) M) produced the strongest inhibitory effect as compared with other purine compounds. N-chloromercuric benzoate and ascorbic acid also inhibited strongly uratoxidase activity. EDTA-Na2, methyl ester of n-oxybenzoic acid, phenyl methyl sulphonyl fluoride and cystein did not influence the enzyme activity.

L4 ANSWER 5 OF 7 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2004234281 EMBASE
TITLE: Biosynthesis of tetrahydrofolate in plants: Crystal structure of 7,8-dihydroneopterin aldolase from Arabidopsis thaliana reveals a novel adolase class.
AUTHOR: Bauer S.; Schott A.-K.; Illarionova V.; Bacher A.; Huber R.; Fischer M.
CORPORATE SOURCE: S. Bauer, Max-Planck-Inst. fur Biochemie, Abteilung Strukturforschung, Am Klopferspitz 18a, D-82152 Martinsried, Germany. stbauer@biochem.mpg.de
SOURCE: Journal of Molecular Biology, (11 Jun 2004) 339/4 (967-979).
Refs: 66
ISSN: 0022-2836 CODEN: JMOBAK
PUBLISHER IDENT.: S 0022-2836(04)00474-7
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Dihydroneopterin aldolase (DHNA) catalyses a retroaldol reaction yielding 6-hydroxymethyl-7,8-dihydropterin, a biosynthetic precursor of the vitamin, tetrahydrofolate. The enzyme is a potential target for antimicrobial and anti-parasite chemotherapy. A gene specifying a dihydroneopterin aldolase from Arabidopsis thaliana was expressed in a recombinant Escherichia coli strain. The recombinant protein was purified to apparent homogeneity and crystallised using polyethylenglycol as the precipitating agent. The crystal structure was solved by X-ray diffraction analysis at 2.2A resolution. The enzyme forms a D(4)-symmetric homooctamer. Each polypeptide chain is folded into a single domain comprising an antiparallel four-stranded .beta.-sheet and two long .alpha.-helices. Four monomers are arranged in a tetrameric ring, and two of these rings form a hollow cylinder. Well defined purine derivatives are found at all eight topologically equivalent active sites. The subunit fold of the enzyme is related to substructures of dihydroneopterin triphosphate epimerase, GTP cyclohydrolase I, and pyruvoyltetrahydropterin synthase, which are all involved in the biosynthesis of pteridine type cofactors, and to **urate oxidase**, although some members of that

superfamily have no detectable sequence similarity. Due to structural and mechanistical differences of DHNA in comparison with class I and class II aldolases, a new aldolase class is proposed.

L4 ANSWER 6 OF 7 MEDLINE on STN
ACCESSION NUMBER: 90151063 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2620492
TITLE: Liver uricase in Camelus dromedarius: purification and properties.
AUTHOR: Osman A M; Del Corso A; Ipata P L; Mura U
CORPORATE SOURCE: Department of Physiology, Faculty of Veterinary Medicine, National University of Somalia, Mogadishu.
SOURCE: Comparative biochemistry and physiology. B, Comparative biochemistry, (1989) 94 (3) 469-74.
Journal code: 2984730R. ISSN: 0305-0491.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19900601
Entered Medline: 19900329

AB 1. Uricase (urate: oxygen oxidoreductase, EC 1.7.3.3) was purified 750-fold from the liver of Camelus dromedarius. 2. The enzyme is a **tetramer** with a Mr of 100,000, displays high specificity for uric acid with a Km of 12 microM and is inhibited by a selected number of purine derivatives carrying oxygen at the C2 position. 3. The effect of pH and the inhibition by thiol compounds and chelating agents on the enzyme activity is reported. 4. Some lines of evidence suggesting the possibility of interaction of camel liver uricase with oligonucleotides are presented.

L4 ANSWER 7 OF 7 MEDLINE on STN
ACCESSION NUMBER: 84256499 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6821367
TITLE: Thermodynamics and stoicheiometry of the binding of substrate analogues to uricase.
AUTHOR: Conley T G; Priest D G
SOURCE: Biochemical journal, (1980 Jun 1) 187 (3) 727-32.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198408
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19980206
Entered Medline: 19840807

AB The subunit composition, metal content, substrate-analogue binding and thermal stability of Aspergillus flavus uricase were determined. A. flavus uricase is a **tetramer** and contains no copper, iron or any other common prosthetic group. Analytical-gel-filtration and equilibrium-dialysis experiments showed one binding site per subunit for urate analogues. The free energy of xanthine binding was -30.5 kJ (-7.3 kcal)/mol of subunit by equilibrium dialysis and -30.1 kJ (-7.2 kcal)/mol of subunit by microcalorimetry. The enthalpy change for xanthine binding was -15.9 kJ (-3.8 kcal)/mol of subunit when determined from the temperature-dependence of the equilibrium constant and -18.0 kJ (-4.3 kcal)/mol of subunit when measured microcalorimetrically. The thermal inactivation rate of A. flavus uricase increases as protein concentration is decreased. This concentration-dependent instability is not due to subunit dissociation.

```
=> s bayol a/au
L5          39 BAYOL A/AU

=> s l5 and urate oxidase
L6           7 L5 AND URATE OXIDASE

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7           4 DUP REM L6 (3 DUPLICATES REMOVED)

=> d l7 1-4 ibib ab

L7   ANSWER 1 OF 4  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
ACCESSION NUMBER:   2002431443  EMBASE
TITLE:              A colorimetric 96-well microtiter plate assay for the
                    determination of urate oxidase activity
                    and its kinetic parameters.
AUTHOR:             Fraisse L.; Bonnet M.C.; De Farcy J.P.; Agut C.; Dersigny
                    D.; Bayol A.
CORPORATE SOURCE:   L. Fraisse, Biochemistry Unit, Sanofi-Synthelabo Recherche,
                    Innopole, Voie No. 1, 31676 Labege Cedex, France.
                    laurent.fraisse@sanofi-synthelabo.com
SOURCE:             Analytical Biochemistry, (15 Oct 2002) 309/2 (173-179).
                    Refs: 23
                    ISSN: 0003-2697  CODEN: ANBCA2
PUBLISHER IDENT.:   S 0003-2697(02)00293-2
COUNTRY:            United States
DOCUMENT TYPE:      Journal; Article
FILE SEGMENT:       029     Clinical Biochemistry
                    037     Drug Literature Index
LANGUAGE:           English
SUMMARY LANGUAGE:   English
AB   Urate oxidase (E.C.1.7.3.3; uricase, urate oxygen
     oxidoreductase) is an enzyme of the purine breakdown pathway that
     catalyzes the oxidation of uric acid in the presence of oxygen to
     allantoin and hydrogen peroxide. A 96-well plate assay measurement of
     urate oxidase activity based on hydrogen peroxide
     quantitation was developed. The 96-well plate method included two steps:
     an incubation step for the urate oxidase reaction
     followed by a step in which the urate oxidase activity
     is stopped in the presence of 8-azaxanthine, a competitive inhibitor.
     Hydrogen peroxide is quantified during the second step by a horseradish
     peroxidase-dependent system. Under the defined conditions, uric acid,
     known as a radical scavenger, did not interfere with hydrogen peroxide
     quantification. The general advantages of such a colorimetric assay
     performed in microtiter plates, compared to other methods and in
     particular the classical UV method performed with cuvettes, are easy
     handling of large amounts of samples at the same time, the possibility of
     automation, and the need for less material. The method has been applied to
     the determination of the kinetic parameters of rasburicase, a recombinant
     therapeutic enzyme. .COPYRGT. 2002 Elsevier Science (USA). All rights
     reserved.

L7    ANSWER 2 OF 4  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
      DUPLICATE 1
ACCESSION NUMBER: 2002-11249  BIOTECHDS
TITLE:            Modification of a reactive cysteine explains differences
                  between rasburicase and Uricozyme (R), a natural Aspergillus
                  flavus uricase;
                     recombinant urate-oxidase and uricase
                     comparison
AUTHOR:           BAYOL A; CAPDEVIELLE J; MALAZZI P; BUZY A; BONNET
                  MC; COLLOC'H N; MORNON JP; LOYAUX D; FERRARA P
```

CORPORATE SOURCE: Sanofi Synthelabo Rech; Sanofi Synthelabo Rech; Univ Caen; Univ Paris 06; Univ Paris 07
LOCATION: Bayol A, Sanofi Synthelabo Rech, Anal and Qual Control Unit, Voie No 1,BP 137, F-31676Labege, France
SOURCE: BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY; (2002) 36, 1, 21-31 ISSN: 0885-4513
DOCUMENT TYPE: Journal
LANGUAGE: English
AB AUTHOR ABSTRACT - **Urate oxidase** is used in humans for the control of uric acid in patients receiving chemotherapy. Rasburicase (Fasturtec(R)/Elitek(R)), a recombinant **urate oxidase** expressed in Saccharomyces cerevisiae, was compared with Uricozyme(R), the natural enzyme produced by Aspergillus flavus. Rasburicase hasa higher purity as demonstrated by SDS/PAGE and chromatographic analysis and a better specific activity. The differences observed for Uricozyme(R) are likely attributable to the previously used purification process, which modifies the enzyme. The production process of rasburicase, on the other hand, preserves the structure of the molecule. MS analysis shows that Uricozyme(R) contains a cysteine adduct on Cys(103). In the crystal structure, the sulphur atom of the cysteine residue in position 103 is orientated to the external surface of the tetramer, whereas the sulphur atom of two other cysteine residues (Cys(35) and Cys(290)) is orientated to the centre of the canal formed by the tetramer. The same adduct is produced by simple incubationof the rasburicase with cysteine.
DERWENT ABSTRACT: The following analytical procedures were used to compare Rasburicase (recombinant **urate-oxidase**) and Uricozyme: isoelectric focusing, SDS-PAGE, size-exclusion chromatography, reverse-phase chromatography analysis, alkylation ofcysteine residues and trypsin (EC-3.4.21.4) digestion, electronspray ionization-mass spectroscopy, HPLC analysis and matrix-assisted laser-desorption ionization-mass spectroscopy, nano-HPLC-Nano-ESI-QTOF sequence analysis and X-ray(11 pages)

L7 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:125778 BIOSIS
DOCUMENT NUMBER: PREV200200125778
TITLE: Stable liquid composition containing **urate oxidase** and lyophilized composition for its preparation.
AUTHOR(S): Aleman, C. [Inventor]; **Bayol, A.** [Inventor]; Breul, T. [Inventor]; Dupin, P. [Inventor]
CORPORATE SOURCE: Montpellier, France
ASSIGNEE: SANOFI
PATENT INFORMATION: US 5811096 Sept. 22, 1998
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Sept. 22, 1998) Vol. 1214, No. 4, pp. 4125. print.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Jan 2002
Last Updated on STN: 26 Feb 2002

L7 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 1995:508505 HCAPLUS
DOCUMENT NUMBER: 122:309587
TITLE: Study of pH and temperature-induced transitions in **urate oxidase** (Uox-EC1.7.3.3) by microcalorimetry (DSC), size exclusion chromatography (SEC) and enzymic activity experiments
AUTHOR(S): **Bayol, A.**; Dupin, P.; Boe, J. F.; Claudy, P.; Letoffe, J. M.
CORPORATE SOURCE: Sanofi Recherche, Labege Innopole Voie n.degree.1, BP 137, Labege, 31376, Fr.
SOURCE: Biophysical Chemistry (1995), 54(3), 229-35

CODEN: BICIAZ; ISSN: 0301-4622
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Purified recombinant **urate oxidase** (urate oxygen oxidoreductase EC 1.7.3.3 re-Uox) has been studied by differential scanning calorimetry (DSC) in correlation with enzymic activity measurements and size exclusion chromatog. Differential scanning calorimetry curves vs. pH show two endothermal effects in the pH range 6-10. The first endotherm reveals a max. stability between pH 7.25 and pH 9.5 corresponding to a temp. of transition Tm1 of 49.0.degree. and an enthalpy of transition of 326 kJ mol-1. This value dramatically decreases below pH 7.25. The behavior of the second endotherm is more complex but the temp. of transition Tm2 is const. between pH 9 and 7.25 and a max. for the corresponding enthalpy is obtained near pH 8 with .DELTA.H2 = 272 kJ mol-1. An optimal pH of 8.0 for the stability of the enzymic activity at elevated temp. was also found which was in good agreement with calorimetric results. Reversibility of the first endotherm is obtained from 20 to 51.5.degree.. The calorimetric result is correlated to enzymic activity, purity by size exclusion chromatog. (SEC) and protein concn. measurements. In contrast, for the second endotherm, after heating up to 68.9.degree., no reversibility was found. Interaction with structural analogs of urate has been studied by DSC. 8-Azahypoxanthine has only a small effect and caffeine has no effect at all. With 8-azaxanthine, a rapid increase of the Tm1 function of the concn. is obtained. At high concn. Tm1 reached the Tm2 value which remained unaffected.

=> d his

(FILE 'HOME' ENTERED AT 16:33:37 ON 26 JUL 2004)

FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, EMBASE' ENTERED AT 16:34:08 ON 26 JUL 2004

L1 0 S URATE OXIDASE IS A TETRAMER
L2 17 S URATE OXIDASE AND TETRAMER
L3 7 DUP REM L2 (10 DUPLICATES REMOVED)
L4 7 FOCUS L3 1-
L5 39 S BAYOL A/AU
L6 7 S L5 AND URATE OXIDASE
L7 4 DUP REM L6 (3 DUPLICATES REMOVED)

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	35.23	35.44

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-1.47	-1.47

STN INTERNATIONAL LOGOFF AT 16:41:04 ON 26 JUL 2004

FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS' ENTERED AT 14:33:50 ON 27 JUL 2004

L1	159	S (URICASE OR URATE OXIDASE) AND (MUTANT? OR VARIANT?)
L2	92	DUP REM L1 (67 DUPLICATES REMOVED)
L3	1	S L2 AND (291 OR 301)
L4	3	S L2 AND ARGININE
L5	0	S L2 AND ARGININE 291
L6	0	S L2 AND THREONINE 301